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QUANTIFYING ENVIRONMENTAL MATERNAL EFFECTS IN THE INVASIVE
PLANT, *SILENE LATIFOLIA*

by

JULIETTE T. JORDAN

(Under the Direction of Lorne M. Wolfe)

ABSTRACT

One of the over-arching goals of biology is to determine the relative importance of nature versus nurture. Nature versus nurture reflects the relative contribution of an individual's genetic makeup and environment to its growth and reproduction. In addition, the environment experienced by an individual's mother can also indirectly affect the performance of her offspring – these are environmental maternal effects (EME). For example, if a mother experiences a high quality environment, her offspring may respond with enhanced growth. We expect natural variation in habitat quality to lead to high levels of EME since mothers will vary in the environment they experience. The goal of my thesis was to quantify EME using a plant species, *Silene latifolia*, which is native to Europe and invasive in North America. I did this by experimentally comparing the amount of variation in life history traits between two generations: Natural (Parent) and Uniform (Offspring). The Parent generation was obtained by collecting seeds from natural populations of Europe and North America (expect high levels of variation). The Offspring generation seeds were created by hand-pollinations from plants growing under uniform greenhouse conditions (expect low levels of variation). Although Parents were only found to be significantly more variable than Offspring for a few life history traits, there was an overall trend in this direction. Therefore, environmental maternal effects are more prevalent in variable habitats. This variation could play a role in *Silene*'s invasion history, enabling the founding population to be better suited for variable environmental conditions.

INDEX WORDS: Maternal effects, Invasive species, Phenotypic plasticity, Genotype and environment interaction, *Silene latifolia*

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by

JULIETTE T. JORDAN

B. S., Georgia Southern University, 2009

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial
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MASTER OF SCIENCE

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DEDICATION

I dedicate this thesis to my parents, Jonas N. and Teresa H. Jordan for all their encouragement and support throughout my life. I owe my academic achievements to them because of all the love, support, and advice they have provided me throughout this chapter of my life.

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CHAPTER I. INTRODUCTION

Overview

Nature poses many challenges to plants and animals. As a result, a major focus of evolutionary ecology has been to understand the sources of variation that allow organisms to succeed. Variation among individuals can be advantageous for population existence in heterogeneous environments (Hoffmann and Merila 1999; Aubin-Horth and Renn 2009; Monty et al. 2009) and is derived from both genetic and non-genetic factors. An individual's genotype can have a profound effect on its performance (Jong 1990; Falconer and Mackay 1996). Yet, genotypes may respond differently to environmental cues even within the same population (Jong 1990; Thompson 1991). Phenotypic plasticity results from the ability of genotypes to express different phenotypes due to environmental conditions (Pigliucci 2001; West-Eberhard 2003; Aubin-Horth and Renn 2009).

Phenotypic plasticity is one form of non-genetic influence on variation. Another non-genetic factor that can cause variation among populations is the environmental conditions experienced by the parents (Falconer and Mackay 1996; Tucic and Avramov 1996). In particular, the maternal environment can have a profound effect on the early juvenile growth and performance due to pre- and post-zygotic influences (Luzuriaga et al. 2005). The influences the maternal environment has on her offspring's phenotype and performance are known as environmental maternal effects (EME). Environmental maternal effects are a response to the past and current environment experienced by the mother and therefore represent a trans-generational phenotypic response to the changing environment (Donohue 1999; Galloway and Etterson 2007; Duckworth 2009; Pfennig and Martin 2009). These environmental maternal effects can also lead to variation among individuals within the same population.

Environmental Maternal Effects in Plants

Environmental maternal effects have been well documented in plants and have been found to be a response not only to environmental conditions but also a response to species interactions such as herbivory or competition (Falconer and Mackay 1996; Roach and Wulff 1987; Mousseau and Fox 1998). Environmental growing conditions of the mother influence

offspring growth and performance. Abiotic factors, such as soil quality and water availability, experienced by the maternal plant can impact seed size. Seed size may be considered a determinate of maternal effects because it is known to be sensitive to the maternal environment (Winn 1991; Fenner 1992; Wolfe 1995; Luzuriaga et al. 2005). There is usually a positive relationship between seed size and seed nutrients, such as content of nitrogen and phosphorus (Vaughton and Ramsey 2001). The maternal environment has been found to be responsible for variation in seed mass and time of germination in plant populations (Byers et al. 1997; Bischoff and Muller-Scharer 2010). Seeds produced by mothers grown in low resource environments have less vigorous growth than seeds produced by mothers grown in high resource environments (Riginos et al. 1984; Aarssen and Burton 1990). Stratton (1989) found that *Erigeron annuus* seeds from mothers grown in high nutrient soils were larger, and in turn grew to be larger seedlings. Larger seeds are more likely to possess a greater amount of resources in the endosperm supplied by the maternal plants enabling them to have greater juvenile growth and a possible advantage in adult growth (Stratton 1989; Vaughton and Ramsey 2001).

Flowering time, as well as time of seed production during the breeding season, can also influence seed size and seedling growth. Seeds of *Hydrophyllum appendiculatum* produced earlier in the breeding season have a greater mass than seeds produced later in the season (Wolfe 1992). Seeds from mothers whose flowers opened earlier in the season were found to germinate earlier than seeds from mothers whose flowers opened later (Galloway 2002). This is most likely due to resource constraints and stresses experienced by the mother later in the season such as changes in climate. Therefore, if the maternal plant developed in an environment with optimal conditions, low stresses, and unrestricted resources her seeds were larger and contained more nutrients for the subsequent growth of the offspring (Mazer and Wolfe 1998; Galloway 2002).

Environmental Maternal Effects in Invasive Species

Evolutionary changes in species, such as their strategy for coping with changing environments, can be difficult to observe in many species since changes in both environmental conditions and the response of the species occur slowly over time. Alien species provide an excellent model to observe evolutionary changes because they have

already been established in a new environment for multiple generations (Sakai et al. 2001; Keller and Taylor 2008; Monty 2009). Invasive species are any species not native to an ecosystem that cause ecological or economical damage (Pimentel et al. 2000; Kolar and Lodge 2001; Lee 2002; Pimentel et al. 2005; Douce et al. 2008). Invasion ecology attempts to study the phenotypic change experienced by invasive species (Elton 1958; Pritchard 1960; Nobel 1989; Blossey and Notzold 1995, Rejmanek and Richardson 1996; Pimentel et al. 2000; Sakai et al. 2001; Keane and Crawley 2002; Blair and Wolfe 2004; Wolfe and Blair 2009). Many factors can lead to an alien species becoming invasive. Invasive species may experience a bottleneck at the time of introduction that decreases the diversity among individuals (Novack and Mack 1993; Blair and Wolfe 2004; Laverghna and Molofsky 2007; Taylor and Keller 2007; Wolfe and Blair 2009). Thus, the founders of the population could be pre-adapted to an aggressive phenotype. In plants, this aggressive phenotype can display increased vigor and may sometimes lead to monocultures (Keane and Crawley 2002). Unfavorable environmental conditions can also lead to the natural selection of phenotypes that can withstand harsh conditions and be advantageous in growth when conditions are favorable (Richards et al. 2006). Genetic differences are not the only factor that drives changes in phenotype. Escaping from species interactions, such as enemy or competition, could also be a factor in invasion success (Blossey and Notzold 1995; Wolfe 2002; Torchin and Mitchell 2004; Morris et al. 2007). The absence of these interactions can allow invaders to allocate resources to growth or reproduction that may have been used previously for protection in their native habitat.

Environmental maternal effects could influence the success of an invader during the time of introduction and throughout colonization. If environmental maternal effects are present in an invasive plant species, the seeds of that population may be more variable. This variation can be beneficial to invaders because it can allow them to cope with unfavorable or favorable environmental conditions (Sakai et al. 2001; Richards et al. 2006). Few studies have examined environmental maternal effects in invasive plant species (see Monty et al. 2009). However, maternal effects may potentially influence spatial dispersal abilities of species experiencing range expansion or successional habitats. In species undergoing range expansion, offspring will likely experience an environment different from the mother, much like an invader (Duckworth 2009).

The ability for a genotype to respond quickly to changes in environmental cues through phenotypic plasticity may also be a key factor in invader success (Funk 2008; Hulme 2008; Monty et al. 2009). Invasive species are believed to be a jack-of-all-trades and have been found to exhibit phenotypic plasticity in their invasive range (Brock et al. 2005; Mal and Lovett-Doust 2005; Richards et al. 2006; Funk 2008). Phenotypic plasticity may likely be the cause of variation present in the invasive plant species (Poulin et al. 2007). Some studies have compared the amount of plasticity present in individuals of the invasive range to those in the native range and found the invasive populations to be more plastic (Lavergne and Molofsky 2007). Chun et al. (2007) compared the plasticity between native and invasive populations of *Lythrum sarucarda*, an aquatic plant native to Europe and invasive to North America. They found that invasive populations were more plastic when water and nutrients were increased. The ability of invasive plants to be phenotypically plastic is advantageous in colonization of competitive environments and may aid in their success as invaders (Lavergne and Molofsky 2007).

Objectives

The main goal of my thesis is to study the variation in phenotype due to maternal effects. Quantifying environmental maternal effects can be challenging, because one must observe the amount of variation in populations independent of genotype. Furthermore, the magnitude of environmental maternal effects may be difficult to interpret due to lack of an appropriate control. My approach was to examine the amount of variation expressed by plants grown from seeds produced in different environments. I used the invasive plant *Silene latifolia* as my study species for this research project. *Silene* is native to Europe, but was introduced to North America in the 1800's and has since become an invasive species. I compared the performance of plants produced in heterogeneous habitats versus homogeneous habitats. The material representing heterogeneous habitats was derived from multiple natural populations: the expectation was that populations from a diversity of locations would differ in some ecological parameters that would result in maternal plants varying in their ability to provision their offspring. The homogeneous plants were produced in the greenhouse where uniform growing conditions would presumably result in less phenotypic variation. This material was produced via a hand-pollination program using some of the field-collected

plants as parents. Thus, this study essentially compared the magnitude of phenotypic variation between the Parent and Offspring generation.

The experimental approach I adopted was to grow seeds from the two generations at the same time over a six-month period and to measure a suite of phenotypic characters including seed mass, germination time, juvenile and adult growth, and longevity. I specifically addressed the following questions:

- 1. Do phenotypic traits differ in magnitude and variance between plants produced by mothers in the field and plants produced by mothers in the greenhouse?** I predicted that environmental maternal effects would be greater in variable habitats (natural populations, Parent generation) than in uniform habitats (greenhouse produced, Offspring generation).

- 2. Do phenotypic traits differ in magnitude and variance between native (European) and introduced (North American) populations?** I predicted that environmental maternal effects would be greater in the introduced (North American) populations than the native (European) populations.

CHAPTER II.

MATERIALS AND METHODS

Description of Study Species

The goal of this research is to use the invasive, dioecious, short-lived perennial, *Silene latifolia*, to quantify environmental maternal effects. *Silene* has emerged as a model species for a variety of studies in evolution and ecology. Furthermore, rapid growth and relatively small adult size make *Silene* easy to grow in experimental settings (Bernasconi et al. 2009). As a result much is known about the life history of *Silene* such as pollination biology (Altizer et al. 1998), disease (Altizer et al. 1998), and sex dimorphism (Delph and Meagher 1995; Carroll and Delph 1996).

Silene is native to Europe (Baker 1947, 1948) and was accidentally transported to North America in the 19th century, most likely through transportation of bulk seed of grains and legumes (Baker 1947, 1948; McNeill 1977). This plant has now spread into all provinces of Canada and the northern two-thirds of the United States (Baker 1948). It is often found in agricultural fields and disturbed areas such as railroads, roadsides, and abandoned lots (Baker 1947, 1948; Taylor and Keller 2007). Since introduction, *Silene* evolved towards a more weedy phenotype. Baker (1965, 1974) describes a weedy plant species as one that exhibits rapid vegetative growth, is able to reproduce both sexual and asexually, is tolerant of varying environmental conditions and having the ability to be phenotypically plastic. Invasive populations of *Silene latifolia* exhibit some of these characteristics through faster germination and flowering time and grow to be overall larger plants (Blair and Wolfe 2004; Wolfe and Blair 2009). Escape from enemies is one process that played a role in *Silene*'s success as an invader. Enemies that were present in its native European range are not present in all populations of North America, such as *Microbotryum violaceum* (anther smut fungus) and *Hadena bicruris* (a moth species with a parasitic larval stage) (Wolfe 2002; Wolfe et al. 2004). By escaping from these enemies, *Silene* can now allocate resources to growth and reproduction that may have been allocated towards protection in its native range (Wolfe and Blair 2009). The individuals that colonized North America may have also been pre-adapted for a weedy phenotype through founder effect or natural selection may have occurred since

introduction that decreased the amount of diversity in North American populations (Taylor and Keller 2007).

Experimental Design

I compared the life history traits of two generations of seeds: Parent and Offspring. The Parent generation seeds were collected from several populations as part of an extensive seed collection of *Silene latifolia* by Dr. Lorne Wolfe and colleagues. To observe environmental maternal effects in a range of environmental conditions, I used six populations of *Silene* seeds collected in the field: three European (Austria, Czech Republic, and the Netherlands) and three North American populations (Illinois, Maine, and North Carolina). Each of the six populations contained one to three families (total number of families =14). A family is defined as seeds produced by the same mother in this study. Half of the Parent generation seeds were planted in Georgia Southern University's greenhouse, grown to flower and cross pollinated within and between families to produce the Offspring generation. I then planted both generations (Parent and Offspring) of seeds together in the greenhouse.

Seeds were planted in a mixture of 50% Metro-Mix ® and 50% potting soil in February 2010 in Georgia Southern University's greenhouse. Two seeds were planted in each pot (n=320 pots/n=640 seeds) and pots were arranged randomly with respect to origin along the greenhouse benches. Pots were observed every day until at least one seed in each pot had germinated. If both seeds germinated the second was removed and replanted in another pot (n=414). Plants were rotated by bench at monthly intervals to control for varying growing conditions in the greenhouse. Seeds were misted three times a day until germination and then watered every other day or as needed. Weeds were removed upon their emergence and Imidicloprid Concentrate 1.47% (Bayer Advanced™ 12 month Tree and Shrub Insect Control Concentrate 2-1-1, Bayer Crop Science LP, Research Triangle Park, NC) diluted to 0.0005% solution was applied once to combat aphids.

My approach to quantify environmental maternal effects was to plant both generations of seeds in two different environments (Figure 1). Maternal effects should add to the amount of phenotypic variation among populations so that when the Parental and Offspring seeds are grown in two environments a wider range of phenotypic expression is

expected. In order to create the two environments, I used two different pot sizes (115mL and 815mL) because *Silene* is known to respond to pot size (Wolfe unpublished data).

Data Collection

I quantified the life history traits of the plants throughout their life cycle by measuring the following traits:

- 1) Seed mass was measured by weighing 10-20 seeds produced by the same mother and father. Individual seeds of *Silene* are very small, so it was necessary to weigh a batch and divide the total by the number of seeds weighed.
- 2) Time of germination was measured from the time seeds were planted to emergence of first true leaves.
- 3) Juvenile growth was measured by counting the number of leaves on each plant biweekly. Because plants did not differ much between biweekly measurements, I decided to use early growth, at 1 month after seeds were planted, and late growth, at four months after seeds were planted, to compare the growth over time.
- 4) Total biomass was determined by harvesting plants at the end of the experiment. The plants were oven dried and weighed to acquire a constant biomass.
- 5) Longevity was determined by the number of days plants lived because some did not live to time of harvest.

Statistical Analysis

All statistical analyses were conducted using JMP (SAS Institute Inc., Version 8.0.1, 2009). Prior to analysis, all data were tested for normality and equal variances. Throughout this thesis, data are presented as non-transformed means \pm standard error. The major focus of this study was to compare the generations and continents, therefore all population data was pooled together and used in subsequent analysis.

Phenotypic Traits

To observe the differences in phenotypic traits between generations or continents I used Student's t-test and mixed-model ANOVAs. The Student's t-tests were used to observe differences in the means of phenotypic traits between the two continents and generations. I

used mixed ANOVAs to determine sources of variation for each phenotypic trait. The ANOVA model I used was Continent, Generation, Continent X Generation.

The growth at early life stages can sometimes influence later life history traits. I used regression analysis to test if early traits influenced subsequent later growth.

Amount of Variation

To compare the amount of variation between continents, I used coefficient of variation to quantify the amount of variation (CV). I chose to use CV because it standardizes the amount of variation to the mean so that traits with differing means can be compared. An increase in CV indicates an increase in amount of variation for that life history trait. The equation for coefficient of variation is:

$$\text{Coefficient of Variation (CV)} = \text{Standard Deviation} \div \text{Mean}$$

The mean of the CV of each population was examined using a Student t-test to observe differences in the amount of variation between the two continents.

Variation between the Parent and Offspring generations was tested using the coefficient of variation. The Student's t-tests was used to compare the difference in the variation of phenotypic traits between the two generations and among continents. I used crossed ANOVAs to determine sources of variation for each phenotypic trait. The ANOVA model I used was Continent, Generation, Continent X Generation.

CHAPTER III. RESULTS

Do phenotypic traits differ in magnitude and variance between plants produced by mothers in the field and plants produced by mothers in the greenhouse?

To quantify maternal effects, I compared life history traits of two different generations of *Silene*: 1) seeds from the field (Parent generation) and 2) seeds from a uniform environment (Offspring generation). I found the Offspring generation exhibited a shorter germination time, grew faster and larger, and lived longer than the Parent generation (Table 1, Figure 2). The interaction of continent X generation was examined; no interaction was found except in germination time and longevity. The means were analyzed for the generations at the continent level (Europe and North America). In the North American populations, the Offspring generation had a larger seed mass, germinated faster and lived longer than the North American Parents (Table 2, Figure 3). The European Offspring germinated faster and grew to be larger plants than European Parents. Early juvenile growth and longevity were greatly affected by seed mass and germination time, but early juvenile growth only had an effect on longevity (Table 3).

Amount of variation in each generation showed that overall traits in the Parent generation tended to be more variable than the Offspring for several traits (Table 4, Figure 4), although only significantly more variable in early juvenile growth and longevity. The interaction of continent X generation was examined; no interaction was found except in longevity. The amount of variation between the two generations analyzed at the continent level (North America and Europe) showed similar results, although the Parent generation was more variable for a greater number of traits. In North American populations, Parents were significantly more variable in only one (longevity) out of six life history traits (Table 5, Figure 5). However, four (all except germination time and late juvenile growth) out of six traits had a trend in the direction of more variation in the Parent generation. Similarly, in European populations, Parents were significantly more variable in two (early juvenile growth and longevity) out of six traits. Yet again, four (all except germination time and late juvenile

growth) out of six traits had a trend of more variation in the Parents than the Offspring generation. Therefore, Parents were more variable than the Offspring in eight of twelve life history traits. The probability of this occurrence is 0.12 (found using binomial distribution) which, although not significant, is lower than the 0.5 occurrence expected.

Do phenotypic traits differ in magnitude and variance between native (European) populations and introduced (North American) populations?

Differences in the phenotypic traits between continents were observed by comparing the means. European seeds were larger than North American seeds, although European seeds germinated more slowly (Table 1, Figure 6). European plants were also found to live longer than North American plants. Although not significant, plants from North America showed a trend of being more variable for all life history traits except total biomass (Table 4, Figure 7).

CHAPTER IV. DISCUSSION

Overview

Many factors can lead to variation in phenotypic traits among populations, one of which is environmental maternal effects. In this thesis, I quantified environmental maternal effects by observing the differences in performance and amount of variation between two generations (Parents: sown in the field and Offspring: sown in the greenhouse) and continents (Native: Europe and Invasive: North America) of the plant, *Silene latifolia*. I found that the Offspring generation exhibited increased performance and variation. My results also showed a trend that North American populations expressed more variation than European populations and performed better than European populations for some traits. This increase in performance and variation may be advantageous in *Silene's* invasion history.

Do phenotypic traits differ in magnitude and variance between plants produced by mothers in the field and plants produced by mothers in the greenhouse?

The Offspring generation was found to have a larger seed mass, however, the amount of variation in seed mass was equal between generations. Seed mass is a good estimate of the maternal resources allocated to the offspring (Wolfe 1995). This quantity of nutrients can vary with the resources available to the mother and can, in turn, have an influence on variation within the population (Vaughton and Ramsey 2001). For example, environmental maternal effects account for at least 20% of the variation in seed mass in *Nemophila menziesii* (Byers et al. 1997). This variation can be due not only to the genotype of the mother but also to varying environmental conditions and the amount of resources available to her. The seeds used in this study were collected from differing habitats that likely varied in a suite of environmental parameters. An increased variation in seed mass was expected in the Parent generation due to the varying environments experienced by the maternal plant, although it was not observed.

Monty et al. (2009) conducted a similar study on environmental maternal effects in invasive species and found that seeds in the controlled environments were more variable. They suggested that this variation could be due to differences in population. Although not

reported in this paper, this study found differences in growth between populations. Because all populations were analyzed together in this study, these differences between populations could explain why the amount of variation did not differ significantly between generations. It should also be noted that seeds from each generation were weighed in sets of 10-20 and an average was calculated. This method may have not been as precise and may have lead to variation in seed mass being over looked.

The offspring generation germinated faster and showed a trend of being more variable in germination time than the Parent generation. This greater variation in the Offspring generation was unexpected. In a similar study conducted by Bischoff and Muller-Scharer (2010) the Parent seeds (collected in the field) and F1seeds (sown in a homogenous environment) were grown together in a controlled environment. They found that F1 seeds had a higher germination percentage in three out of the four species they observed. They also found both generations to be variable for germination time, although the Parent generation in their study still exhibited greater variation. Variability in seed dormancy or germination time can be favorable in natural populations because it reduces the risk of all offspring experiencing competition or other unfavorable environmental conditions (Venable 1985; Clauss and Venable 2000; Luzuriaga et al. 2005). Thus, more research should be conducted to understand why this variation is present in the Offspring generation.

Although the Offspring generation had a larger seed mass and germinated faster, a regression showed that seed mass did not influence germination time. Therefore, the large seed mass expressed in the Offspring may not have been due to the amount of nutrients contained in the endosperm, which would likely have influenced germination to occur more quickly. There are other seed traits besides amount of nutrients that can influence seed mass, such as seed coat thickness (Luzuriaga et al. 2005). If the seed coat is thicker then there might be a delay in germination time.

Early juvenile growth was found to be more variable in the Parent generation. This result was expected due to the fact that environmental maternal effects have been found to be more prevalent early in the life cycle and to diminish in later life history traits (Roach and Wulff 1987). Later growth and reproduction can be influenced by other variables such as the present environment the offspring is experiencing and the offspring's own genotype (Roach and Wulff 1987; Wolfe 1995; Hereford and Moriuchi 2005; Bischoff and Muller-Scharer

2010). In this study, both late juvenile growth and total biomass were more variable in the Offspring generation than in the Parent generation. However, longevity was found to have significantly more variation in the Parent generation than the Offspring. Early juvenile growth and longevity were both influenced by seed mass and germination time. Maternal effects through seed mass or quality could possibly directly influence these later life history traits. The endosperm, which contains the nutrients for the seed and effects early plant growth, is highly influenced by the maternal environment (Winn 1991; Fenner 1992; Wolfe 1995; Luzuriaga et al. 2005). Therefore, if more resources are available to the seed or seedling the plant may, in turn, grow larger.

When generations from each continent were analyzed separately, similar results occurred. The European Parent generation was more variable than the European Offspring generation for early juvenile growth, however, no significant difference was observed between the North American generations for this trait. North America is the introduced range for *Silene* in which increased maternal effects would be expected. Environmental maternal effects may have been present in the founding individuals of the invasive populations, but are no longer present due to environmental or genetic changes that occurred after introduction. With future research, environmental maternal effects may be more evident in *Silene*.

Do phenotypic traits differ in magnitude and variance between native (European) populations and introduced (North American) populations?

European plants had larger seeds, germinated more slowly and lived longer. European plants have been found in past studies to germinate more slowly, but North American plants were larger as juveniles and flowered earlier (Blair and Wolfe 2004; Wolfe and Blair 2009). This study did not observe differences in flowering time and found no difference in juvenile size between the two continents, but there was a trend of North American plants exhibiting a larger total biomass.

North American populations in this study showed a trend of exhibiting more variation in phenotypic growth than European populations for some traits. This does not coincide with past research in which genetic variation in invasive populations of *Silene* were found to be low due to the occurrence of a bottleneck at the time of introduction (Taylor and Keller 2007). However, the increase in phenotypic variation in the invasive populations may have

arisen after the bottleneck through natural selection or it may be due to multiple introductions (Taylor and Keller 2007). Natural selection has a stronger influence on phenotypic variation than genetic drift in other invasive species (Laverge and Molofsky 2007), so the amount of phenotypic variation present in the introduced populations of *Silene* could also be due to natural selection through escape from enemies or unfavorable conditions. By escaping from a natural enemy in its native land, *Silene* is now able to allocate resources to growth that may have previously been allocated to protection (Wolfe 2002; Wolfe et al. 2004). Similarly, unfavorable conditions experienced by *Silene* may have led to selection of individuals that can withstand harsh conditions and flourish in favorable environmental conditions (Richards et al. 2006). Both of these phenomena may lead to increasing phenotypic variation in performance and growth.

Implications and Future Work

Environmental maternal effects influence performance and phenotypic expression in offspring. I found that environmental maternal effects are not only responsible for variation in growth but also for performance. Seed mass can be an estimate of environmental maternal effects due to resources contained in the endosperm and can subsequently affect later plant growth (Winn 1991; Fenner 1992; Wolfe 1995; Luzuriaga et al. 2005). Because environmental conditions may differ among populations, variation among seeds and other life history traits may be expressed. Therefore, the maternal environment can influence the overall growth of the offspring and the amount of variation present in populations. In the future, this work may be better conducted in other environments rather than just the greenhouse. Seeds grown in the same soil as their maternal plant grew to be larger plants than seeds grown in the soils not of their mother (Hereford and Moriuchi 2005; Galloway and Etterson 2007; Bischoff and Muller-Scharer 2010). One could then compare differences in other phenotypic traits expressed in conditions similar to and different from the mother.

There are many factors that can influence the success of invaders such as escape from enemies and phenotypic plasticity. Environmental maternal effects could also be a contributor to their success by the production of high quality and variable seeds. My study found that environmental maternal effects are more prevalent in individuals produced by mothers that grew in variable habitats. Maternal effects allow a response to unfavorable

environmental conditions. Invasive species might favor high levels of phenotypic variation as an adaptation. Therefore, the added variation from environmental maternal effects could be advantageous to a recent invader. If seeds from multiple or heterogeneous populations are the founding individuals in a new habitat, there may be more variation among these individuals. Thus, it is more likely that a subset of these seeds will be able to withstand the new and possibly unfavorable conditions, aiding them in survival in their new land and increasing their ability to colonize.

Table 1. Analysis of variance table of the means for all life history traits of *Silene latifolia*. Continent (Europe or North America) and Generation (Parent or Offspring).

Source	DF	Seed Mass	Germination Time	Early Juvenile Growth	Late Juvenile Growth	Total Biomass	Longevity
Continent	1	63.05***	5.158*	3.352	0.065	0.267	7.365**
Generation	1	22.984***	25.023***	5.092*	5.095*	6.153*	66.420***
Continent X Generation	1	0.121	5.328*	2.899	0.054	0.1187	10.203**

*P< 0.05, **P< 0.01, ***P< 0.0001

Table 2. Table of t-test values with means between generations of both continents (Europe and North America) of *Silene latifolia*.

Source	DF	Europe	North America
Seed Mass	1	0.034	9.131**
Germination Time	1	4.356*	21.431***
Early Juvenile Growth	1	0.37	6.219*
Late Juvenile Growth	1	2.247	1.307
Total Biomass	1	3.973	2.423
Longevity	1	13.645**	78.279***

*P< 0.05, **P< 0.01, ***P< 0.0001

Table 3. Relationship between seed mass and life history traits of *Silene latifolia*.

Source	DF	r² -value
Seed Mass X Germination Time	1	0.001
Seed Mass X Early Juvenile Growth	1	0.022*
Seed Mass X Late Juvenile Growth	1	0.008
Seed Mass X Total Biomass	1	0.013
Seed Mass X Longevity	1	0.027**
Germination Time X Early Juvenile Growth	1	0.053***
Germination Time X Late Juvenile Growth	1	0.004
Germination Time X Total Biomass	1	0.0002
Germination Time X Longevity	1	0.048***
Early Juvenile Growth X Late Juvenile Growth	1	0.001
Early Juvenile Growth X Total Biomass	1	0.002
Early Juvenile Growth X Longevity	1	0.152***

*P< 0.05, **P< 0.01, ***P< 0.0001

Table 4. Analysis of variance table for coefficient of variation for life history traits between generations of *Silene latifolia*.
Continent (Europe or North America) and Generation (Parent or Offspring).

Source	DF	Seed Mass	Germination Time	Early Juvenile Growth	Late Juvenile Growth	Total Biomass	Longevity
Continent	1	0.221	0.156	1.918	0.042	0.114	33.185**
Generation	1	1.010	6.506	24.798**	14.023*	3.383	121.731***
Continent X Generation	1	0.347	0.064	0.190	2.638	1.185	27.230**

*P< 0.05, **P< 0.01, ***P< 0.0001

Table 5. Table of t-test values with coefficient of variation between generations of both continents (Europe and North America) of *Silene latifolia*.

Source	DF	Europe	North America
Seed Mass	1	0.123	2.242
Germination Time	1	6.164	1.969
Early Juvenile Growth	1	16.481**	6.312
Late Juvenile Growth	1	0.521	2.745
Total Biomass	1	0.315	3.743
Longevity	1	18.218**	333.60***

*P< 0.05, **P< 0.01, ***P< 0.0001

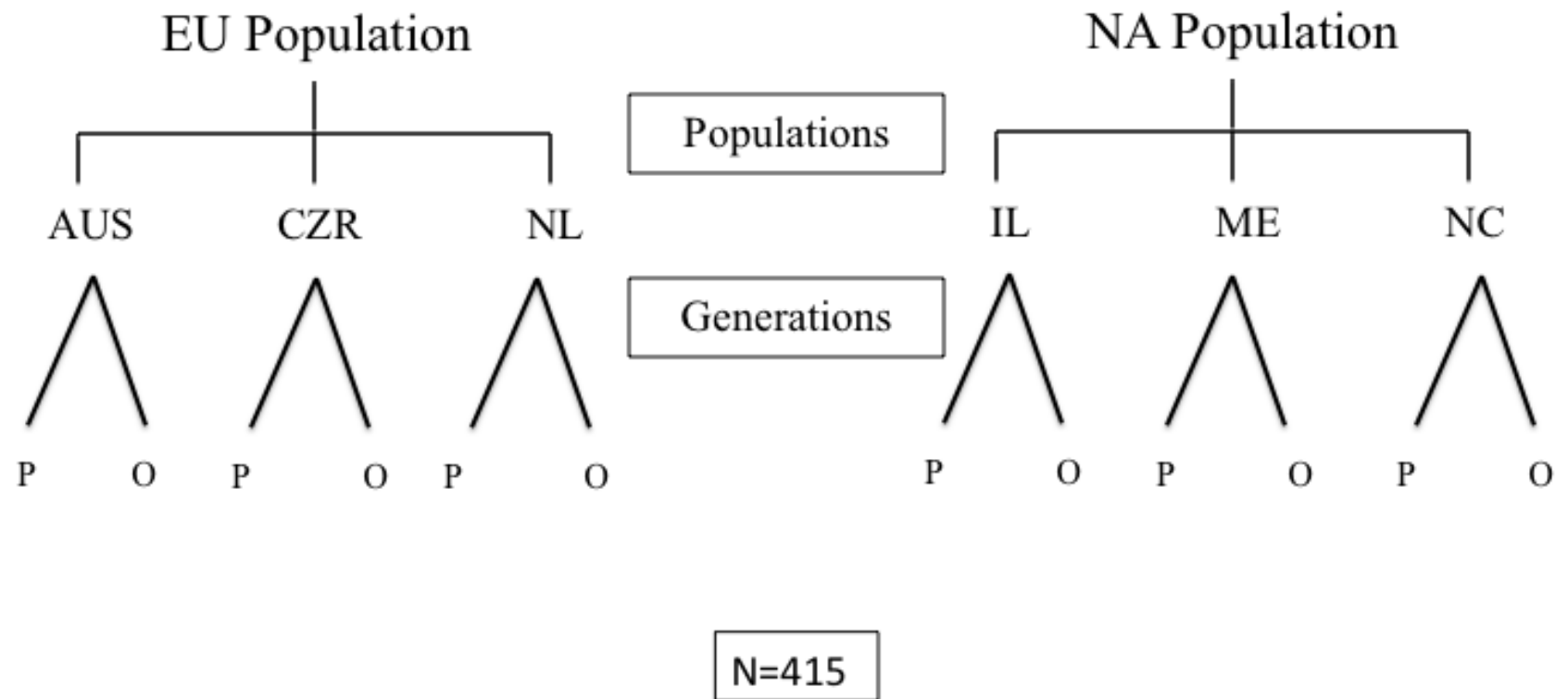


Figure 1: The experimental design of the study investigating environmental maternal effects. Three populations from each continent (six populations total). Each population contained two generations (P=Parent and O=Offspring). Each generation was planted in two different sized pots.

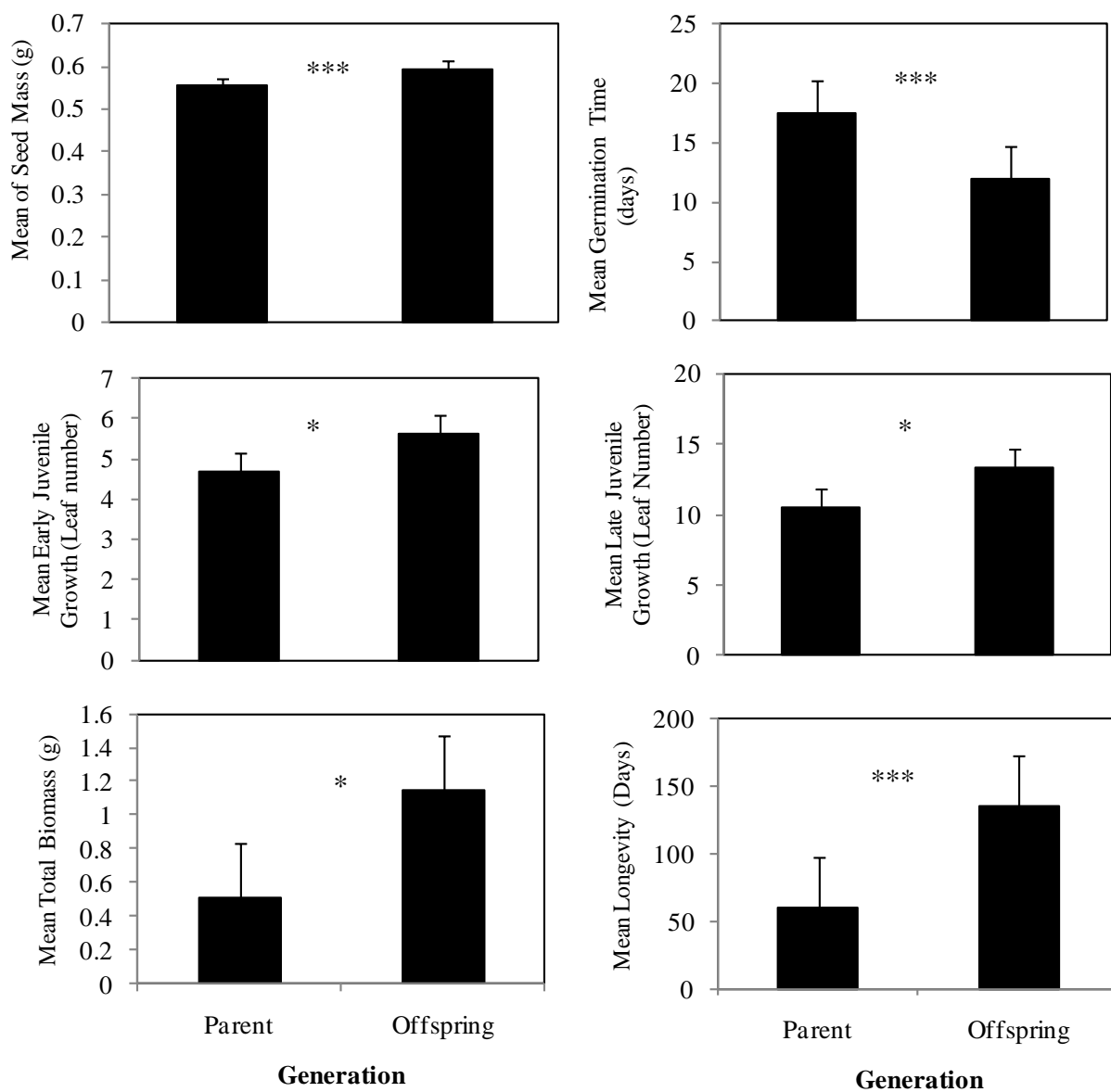


Figure 2: The means of both generations (Parent and Offspring) of *Silene latifolia*. Values = mean \pm SE and were calculated from ANOVA. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

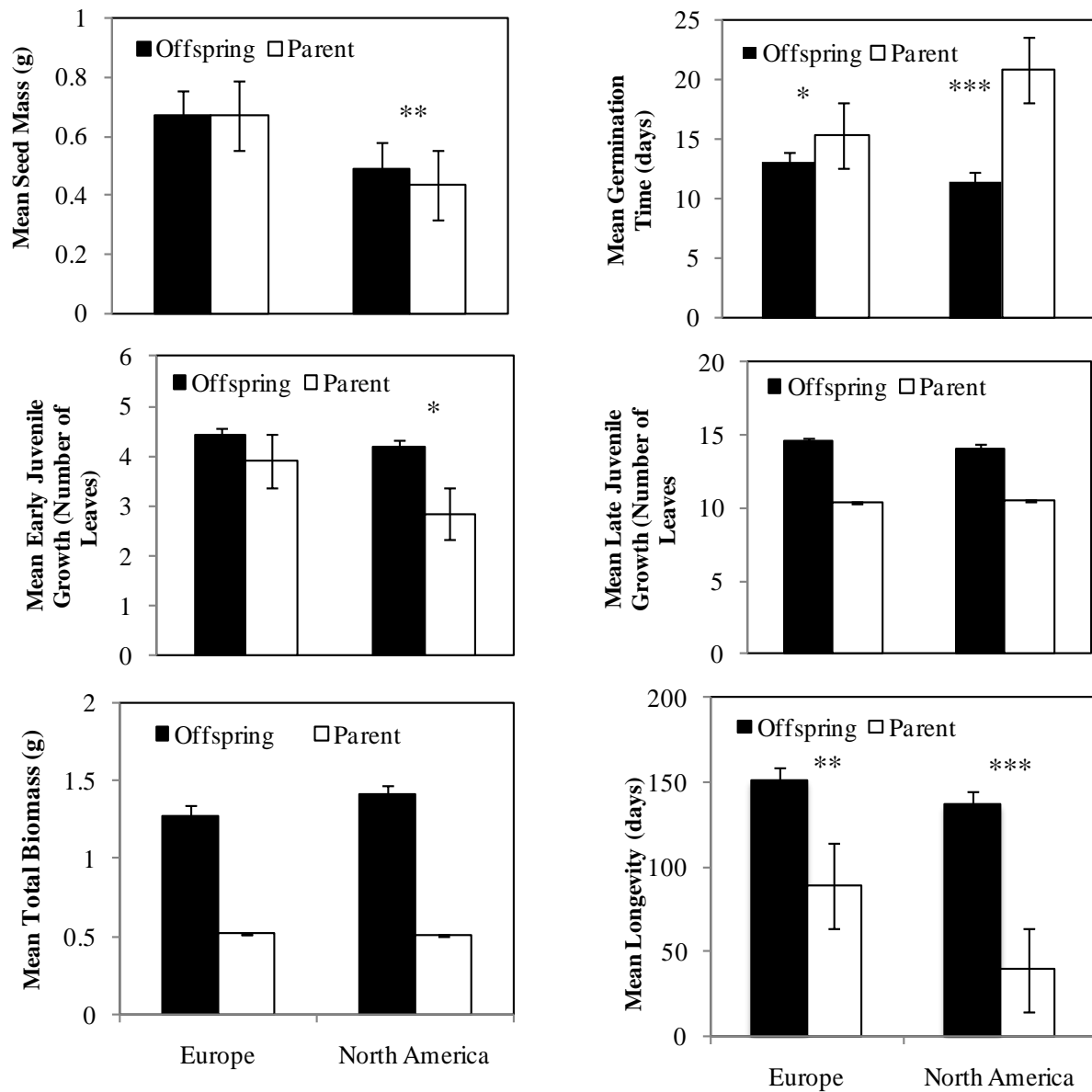


Figure 3: The mean for both generations (Parent and Offspring) of *Silene latifolia* for both continents. Values = mean \pm SE and were calculated from Student's t-test (*P < 0.05, **P < 0.01, ***P < 0.0001).

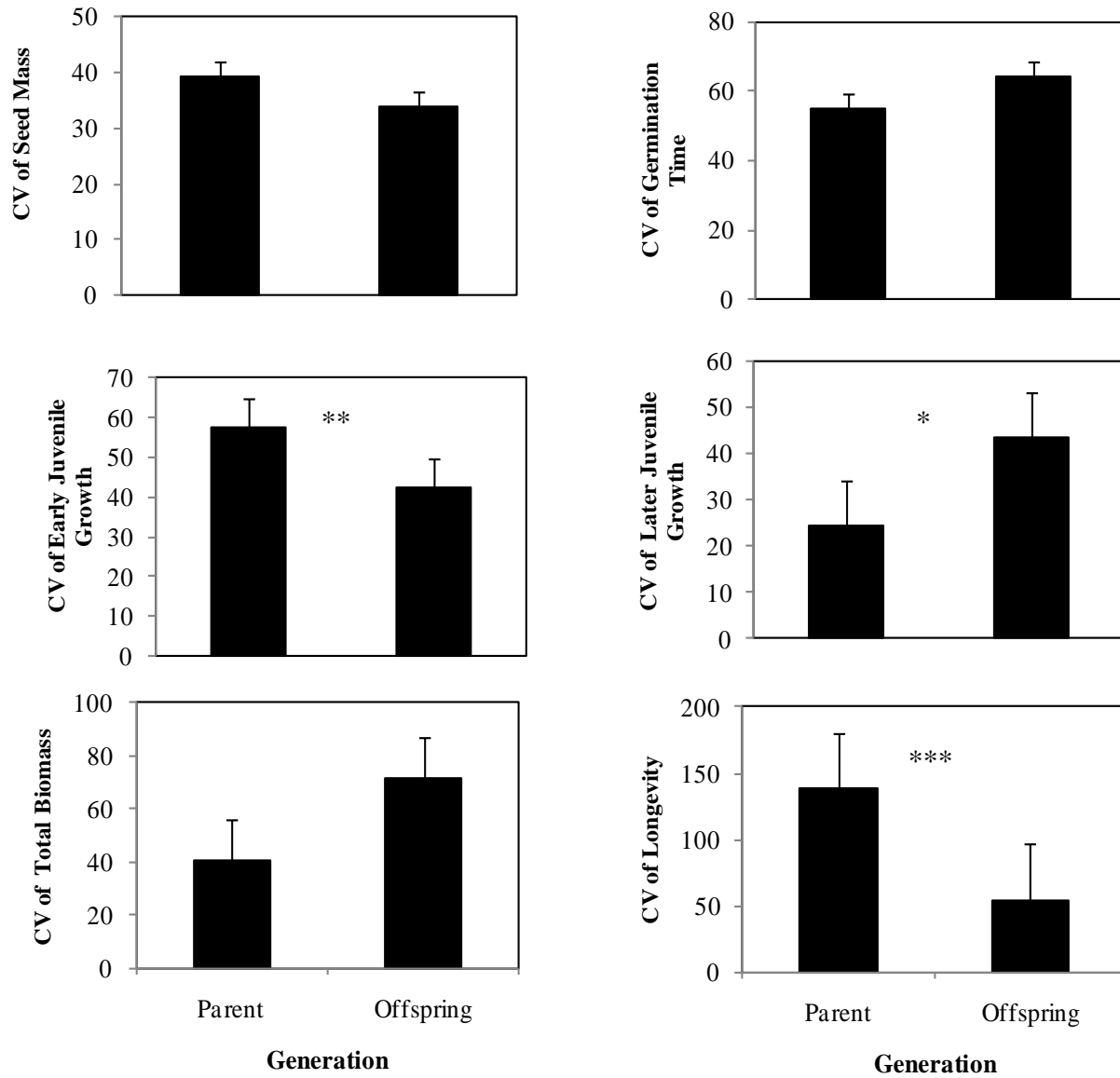


Figure 4: The mean of coefficient of variation of both generations (Parent and Offspring) of *Silene latifolia*. Values = mean \pm SE and were calculated from ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

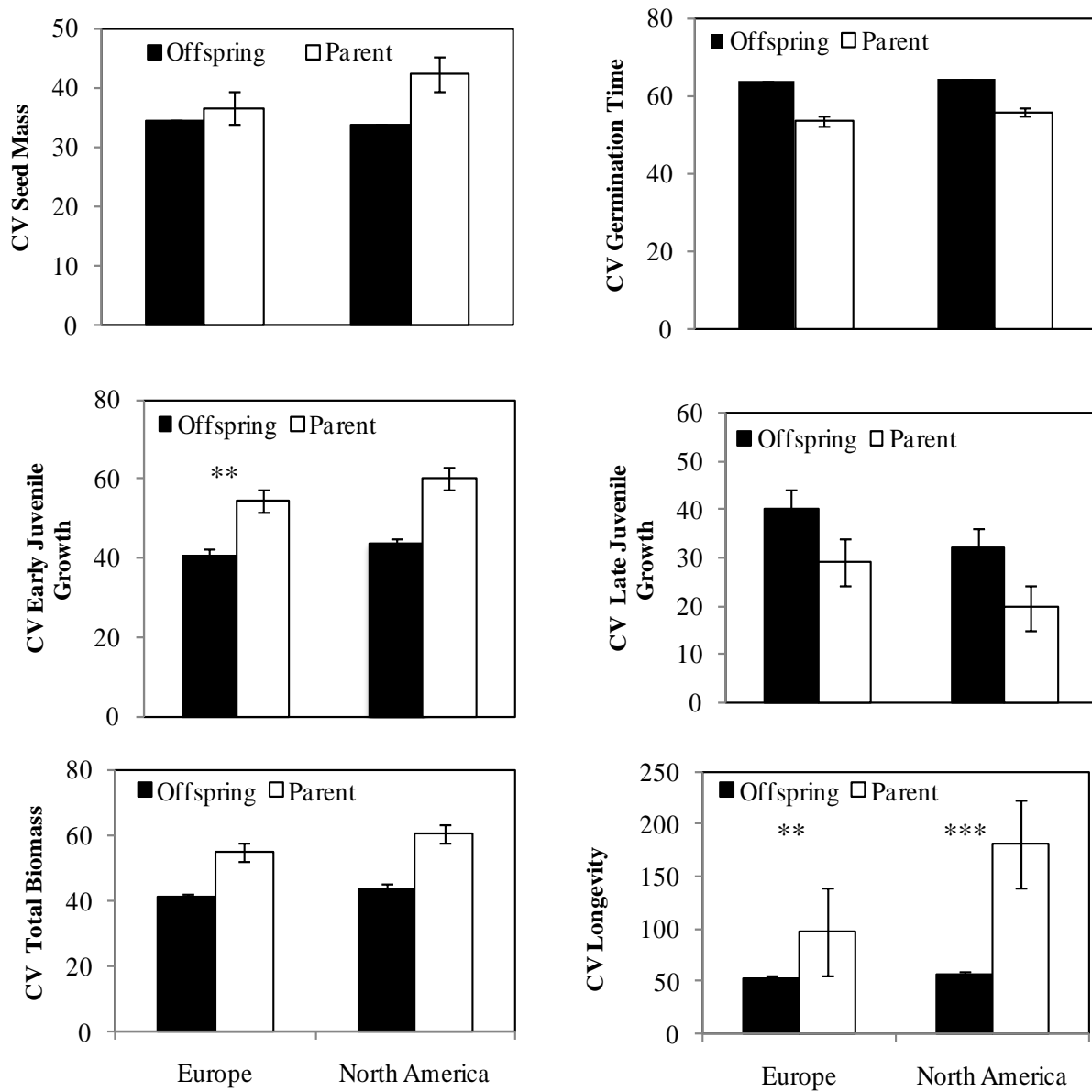


Figure 5: The mean of coefficient of variation for both generations (Parent and Offspring) of *Silene latifolia* for both continents. Values = mean \pm SE and were calculated from Student t-test (*P < 0.05, **P < 0.01, ***P < 0.0001).

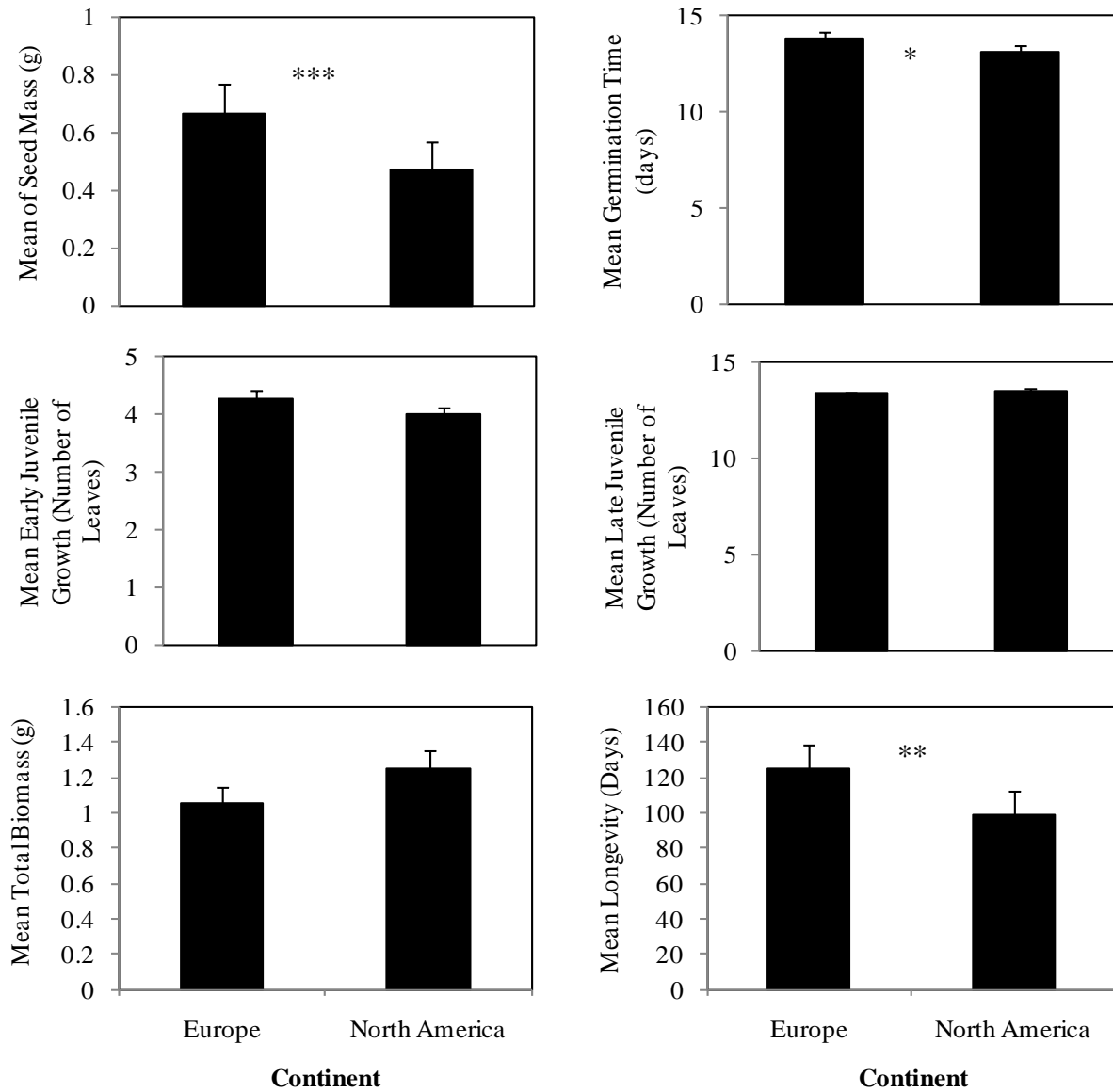


Figure 6: The means of both continents (Europe and North America) of *Silene latifolia*.

Values = mean \pm SE and were calculated from ANOVA (*P < 0.05, **P < 0.01, ***P < 0.0001).

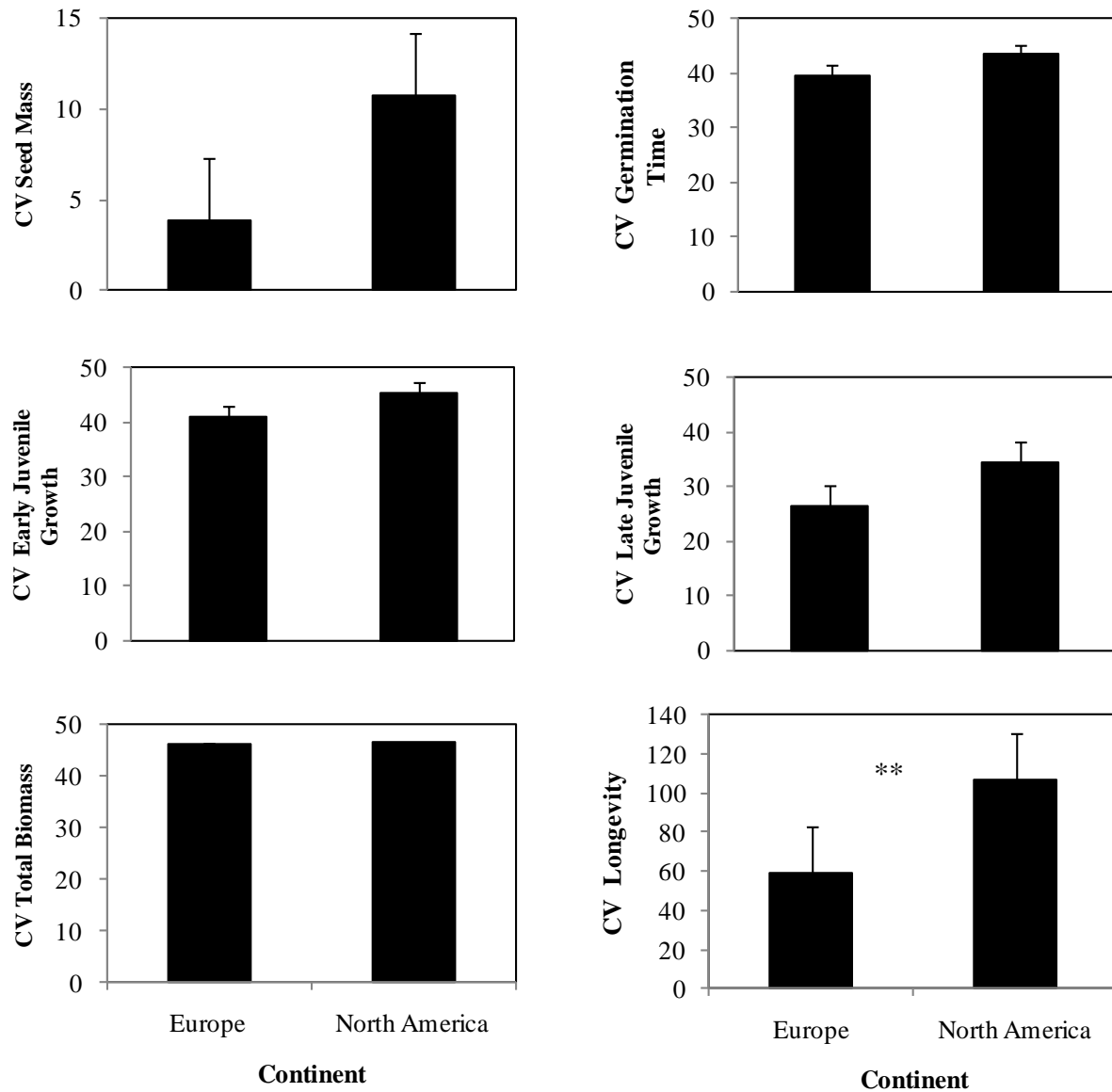


Figure 7: The mean of coefficient of variation of both continents (Europe and North America) of *Silene latifolia*. Values = mean \pm SE and were calculated from ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

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APPENDICES

Appendix A. Results and Discussion for Reaction Norms data

RESULTS

Although pot size did not have an effect on germination time, at every other life stage plants in large pots were found to grow larger than plants grown in smaller pots (Table 7, Figure 8). However, no difference was found in the population X pot size interaction or continent X pot size interaction.

DISCUSSION

Plants grown in large pots were found to grow larger than plants in small pots. This is most likely due to a greater amount of resources available. Therefore, *Silene* does exhibit phenotypic plasticity. However, no differences were found between the population X pot size interaction or continent X pot size. Therefore, no genotype X environment interaction was found.

Table 7. Analysis of variance table for reaction norms of all life history traits of *Silene latifolia*. Continent (Europe or North America), Population (Austria, Czech Republic, Netherlands, Illinois, Maine, or North Carolina) and Pot Size (Large or Small).

Source	Germination Time	Early Juvenile Growth	Late Juvenile Growth	Total Biomass
Continent	5.158*	3.3522	0.065	7.3649*
Population [Continent]	0.238	1.4797	1.473	1.7454
Pot Size	0.990	9.8311*	11.188**	0.2794
Continent X Pot Size	1.394	0.7860	0.190	1.0494
Generation X Pot Size	2.924	2.5322	2.733	0.0007
Population	-	0.8936	1.020	1.5107
Population X Pot Size	-	2.1740	1.141	1.4536

*P< 0.05, **P< 0.01, ***P< 0.0001

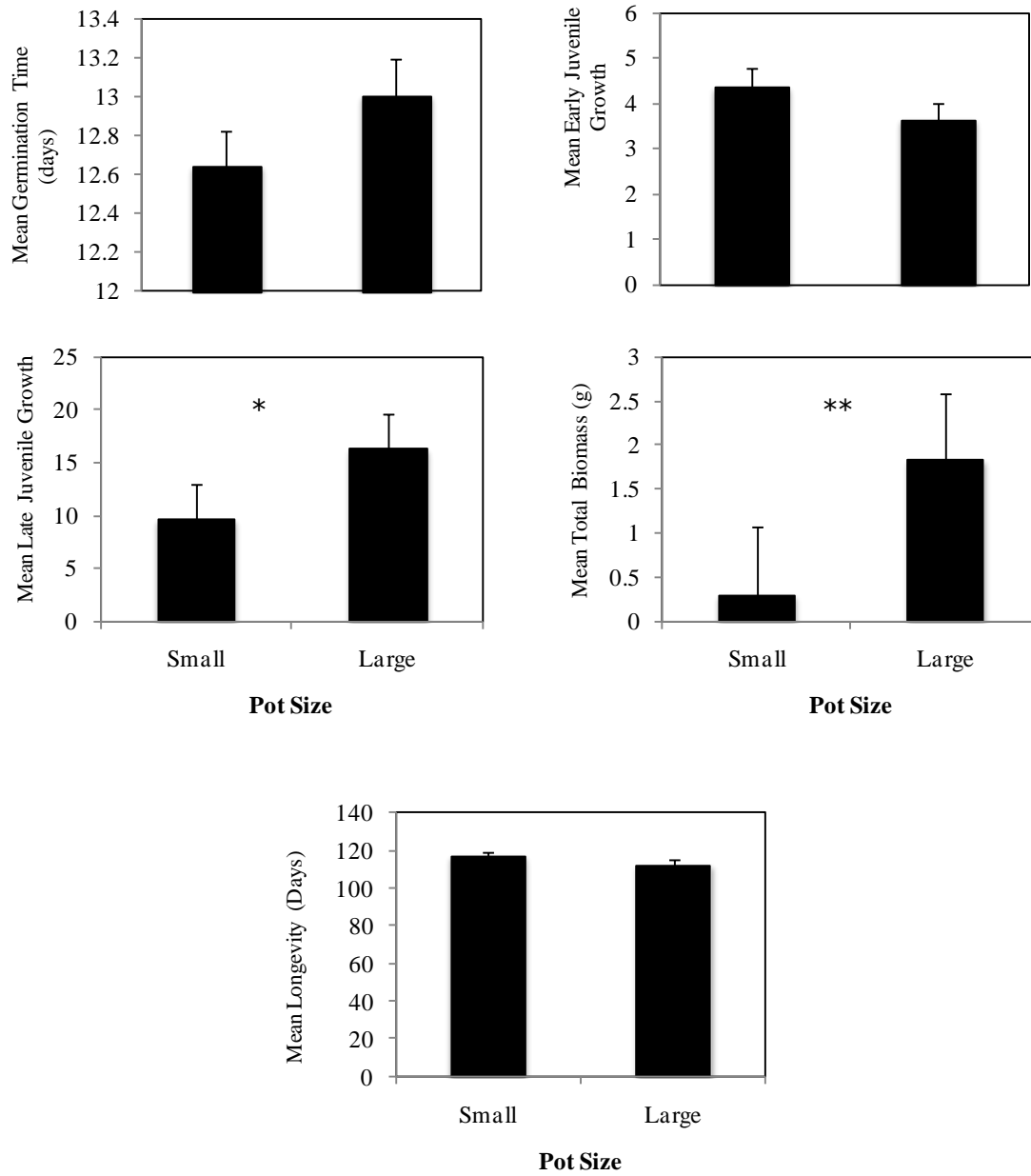


Figure 8: The mean of both pot sizes (Large and Small) of *Silene latifolia*. Values = mean \pm SE and were calculated from ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

Appendix B. Results and Discussion of Inbreeding Depression data.

RESULTS

Inbreeding Depression was found to be most prevalent in later life stages especially total biomass (Table 8, Figure 9). Inbred individuals were found to have a larger seed mass than their outcrossed counter parts (Table 8, Figure 9). Yet, in all later life stages outcrossed individuals performed better than inbred individuals.

Two-way ANOVAs were also used to observe the interaction between breeding system and continent. The continent X breeding system interaction was found to have an effect on seed mass where European plants produced larger seeds than North American plants (Table 8, Figure 9).

DISCUSSION

The outcrossed individuals were found to perform much better than the inbred individuals in the later life history traits. A similar study conducted by Teixeira et al. (2009) found that inbred offspring, when compared to outcrossed offspring in *Silene latifolia*, had the greatest effects on growth, age at first flowering time, and overall fitness. Another study conducted by Richards (2000) on inbreeding depression in *Silene latifolia* found that seeds produced between siblings were 60% less likely to germinate than outcrossed seeds. The results of this study coincide with both of the findings of those past experiments.

Since inbreeding depression was not observed until later life stages, environmental maternal effects could be covering inbreeding depression in the early life stages. Therefore, the phenotype expressed in later life stages more than likely represent the individuals' own genotype and environment interaction. Wolfe (1993) found that *Hydrophyllum appendiculatum* did not express a decrease in vigor due to inbreeding depression until the second year. During the second year of the life cycle, inbred offspring were found to be 22% smaller than their outcrossed counterparts. This result is most likely due to the decrease in maternal effects after the first year of life.

Table 8. Analysis of variance table for inbreeding depression of all life history traits of *Silene latifolia*. Continent (Europe or North America), Population (Austria, Czech Republic, Netherlands, Illinois, Maine, or North Carolina) and Breeding System (Inbred or Outcrossed).

Source	Seed Mass	Germination Time	Early Juvenile Growth	Late Juvenile Growth	Total Biomass	Longevity
Continent	63.05***	5.158*	3.3522	0.0653	7.3649**	7.3649**
Population [Continent]	50.772***	0.238	1.4797	1.4733	1.7454	1.7454
Breeding System	13.991**	0.215	2.6546	3.6915	5.9587*	3.4531
Continent X Breeding System	7.776**	2.446	0.3829	0.2927	1.7106	2.1524
Pot Size X Breeding System		0.202	0.0044	6.2629*	5.3172*	0.1355

*P< 0.05, **P< 0.01, ***P< 0.0001

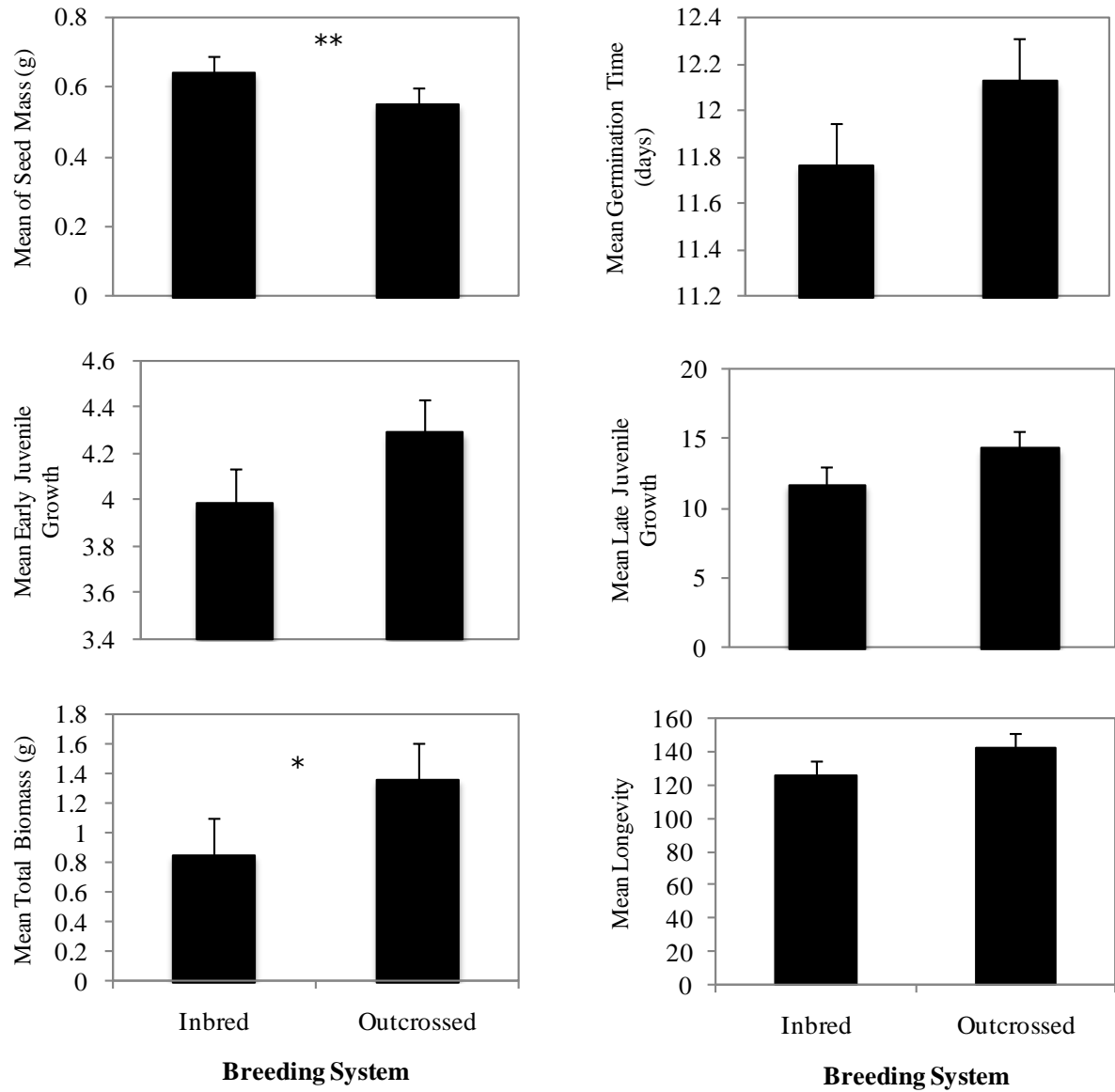


Figure 9: The mean of both breeding systems (Inbred and Outcrossed) of *Silene latifolia*.

Values = mean \pm SE and were calculated from ANOVA (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

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